Lyme Borreliosis in Canada: Biological Diversity and Diagnostic Complexity from an Entomological Perspective

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Lyme borreliosis in Canada: biological diversity and diagnostic complexity from an entomological perspective

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Abstract—Lyme borreliosis (LB), also known as Lyme disease, is emerging as a serious tick-borne illness across Canada. More than three decades of research on LB in North America and Europe have provided a large, complex body of research involving well-documented difficulties at several levels. However, entomologists are well situated to contribute to resolving some of these challenges. The central pathogen in LB, the spirochete *Borrelia burgdorferi* Johnson *et al*., includes numerous genospecies and strains that are associated with different disease symptoms and distributions. The primary vectors of LB are ticks of various *Ixodes* Latreille species (Acari: Ixodida: Ixodidae), but questions linger concerning the status of a number of other arthropods that may be infected with *B. burgdorferi* but do not transmit it biologically. A variety of vertebrates may serve as reservoirs for LB, but differences in their ability to transmit LB are not well understood at the community level. Persistent cystic forms of and immune-system evasion by *B. burgdorferi* contribute to extraordinary challenges in diagnosing LB. Multiple trade-offs constrain the effectiveness of assays like ELISA, Western blot, polymerase chain reaction, and microscopic visualization of the spirochetes. Consequently, opportunities abound for entomologists to contribute to documenting the diversity of the players and their interactions in this devilishly complex disease.

Résumé—La borréliose de Lyme (LB), connue aussi sous le nom de maladie de Lyme, est en train de devenir une importante maladie transmise par les tiques dans l'ensemble du Canada. Les recherches au cours de plus de trois décennies sur LB en Amérique du Nord et en Europe ont fourni un ensemble considérable et complexe de travaux qui comporte des problèmes bien identifiés à différents niveaux. Les entomologistes sont, cependant, bien placés pour contribuer à solutionner certains de ces défis. Le pathogène principal de LB, le spirochète *Borrelia burgdorferi* Johnson *et al*., englobe plusieurs espèces génétiques et souches qui sont associées à des symptômes et des répartitions différentes de la maladie. Les vecteurs principaux de LB sont des tiques de différentes espèces d'*Ixodes* Latreille (Acari: Ixodida: Ixodidae), mais il reste des questions concernant le statut de plusieurs autres arthropodes qui peuvent être infectés par *B. burgdorferi*, mais qui ne le transmettent pas biologiquement. Divers vertébrés peuvent servir de réservoirs pour LB, mais les différences relatives dans leur capacité à transmettre LB ne sont

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pas bien comprises au niveau de la communauté. Les formes kystiques persistantes et l'évasion du système immunitaire chez *B. burgdorferi* rendent le diagnostic de LB extraordinairement compliqué. De nombreux compromis limitent l'efficacité de tests biologiques, tels que la méthode ELISA, le buvardage western, l'amplification en chaîne par polymérase et la visualisation des spirochètes au microscope. En conséquence, il existe de multiples occasions pour les entomologistes de contribuer à l'étude de la diversité des intervenants et de leurs interactions dans cette maladie diablement complexe.

[Traduit par la Rédaction]

Introduction

Lyme borreliosis (LB) is a potentially debilitating illness that is typically vectored by ixodid ticks (Acari: Ixodida: Ixodidae) and caused by infection by the spirochete *Borrelia burgdorferi* Johnson *et al.* or other closely related species of *Borrelia* Swellengrebel. While the disease is also known as Lyme disease, the term Lyme borreliosis is becoming increasingly prominent internationally, to emphasize the causative organism (Gray *et al.* 2002). It has become the most common disease vectored by arthropods in the United States of America (USA), with the majority of cases occurring in the northeastern and north-central regions. In 2005, a total of 12 914 cases were reported among the 56 million inhabitants of the 10 states that border eastern and central Canadian provinces, and the incidence of LB has more than doubled since it became nationally notifiable in the USA in 1991 (Centers for Disease Control and Prevention (CDC) 2007). In contrast, although most of the 32 million inhabitants of Canada lived within 150 km of the United States border in 2005, fewer than 50 cases of LB per year were diagnosed in humans by Canadian laboratories and approximately half of these were associated with travel to areas outside Canada (Charron and Sockett 2005).

In Canada, accurate LB statistics have been difficult to obtain because the disease is not yet nationally notifiable (Ogden *et al.* 2008*a*; Canadian Paediatric Society Infectious Diseases and Immunization Committee (CPS) 2009). Nonetheless, *B. burgdorferi* has now been recognized in every province (Table 1). Recent passive surveillance from Manitoba eastward has shown that 12.5% of sampled specimens of the tick vector *Ixodes scapularis* Say were infected with *B. burgdorferi*, including 10% of those collected on humans (Ogden *et al.* 2006). Infected ticks are not a new phenomenon in Canada; soon after the original description of *B. burgdorferi*, Lindsay *et al.* (1991) reported a 58% prevalence of *B. burgdorferi* in adult *Ixodes* Latreille at Long Point, Ontario.

LB has been recognized by the Public Health Agency of Canada as becoming increasingly important for Canadians (Ogden *et al.* 2008*a*, 2008*d*). Even so, some infectious-diseases specialists disagree, noting that the disease is rare and reports of endemic cases have not increased in Canada during the past two decades (CPS 2009). In the USA, controversy over the diagnosis and treatment of LB has resulted in an increasing polarization of views, with some doctors interpreting it as a serious illness with long-term sequelae (*e.g.*, Fallon *et al.* 2008; Stricker and Johnson 2008; Cameron 2009) and others viewing it as a faddish ailment used by patients to displace the less socially palatable medical explanation of psychiatric difficulties (Sigal and Hassett 2002; Hassett *et al.* 2008). Ballantyne (2008) concluded that such controversies can only be resolved with further research. Limitations in the sensitivity and specificity of blood tests across the genetic diversity of *Borrelia* species, an incomplete understanding of their immune-system evasion and persistence, variation in the degree of human immunity, and complications due to co-infections by other disease-causing microorganisms all contribute to the complexity of the interactions between the spirochetes, their vectors, and their vertebrate hosts. Even in Connecticut, where LB was first described and incidence is still very high, treatment has been sufficiently controversial that the Connecticut General Assembly recently passed legislation protecting doctors from disciplinary action for prescribing long-term treatment with antibiotics (Connecticut General Assembly 2009).

Table 1. Reports of Lyme borreliosis (LB) and *Ixodes* ticks in Canada.

	First report of human LB in *Canada Diseases Weekly Report*	First report of *I. scapularis* or *I. pacificus*	PCR (or IFA) positive for *Borrelia burgdorferi* in ticks	Positive cases for dogs in 2007[1]
Nova Scotia	—	1984[2]	1999[3]	34
New Brunswick	1987[4]	1990[2]	1996[5] (IFA)	11
Newfoundland	1989[4]	1994[6]	2001[7] (*B. burgdorferi*); 2006[8] (*B. garinii*)	n/a
Prince Edward Island	—	1989[2]	1991[9] (IFA)	1
Quebec	1984[10]	1989[2]	1996[11]	65
Ontario	1977[10]	1904[12]	1988[13] (IFA), 1993[14]	395
Manitoba	1988[4]	1989[2]	2002[15]	229
Saskatchewan	1999[16]	1998[16]	—	3
Alberta	1989[4]	1998[3]; 2002[15]	1994[17]	5
British Columbia	1988[4]	1910[18]	1993[19]	4

Note: Sources are as follows: 1, IDEXX Laboratories, Inc. (2008); 2, Costero (1990); 3, Scott *et al*. (2001); 4, Mackenzie (1990); 5, Bjerkelund (1997); 6, Artsob *et al*. (2000); 7, Whitney (2005); 8, Smith *et al*. (2006); 9, Artsob *et al*. (1992); 10, Bollegraaf (1988); 11, Louise Trudel, Laboratoire de santé publique du Québec, Institut national de santé publique du Québec, Sainte-Anne-de-Bellevue, personal communication, 26 March 2009; 12, Nuttall and Warburton (1911); 13, Barker *et al*. (1988); 14, Banerjee *et al*. (1995*b*); 15, Morshed *et al*. (2005); 16, Lindsay et al. (1999*c*); 17, Banerjee *et al*. (1995*a*); 18, Gregson (1956); 19, Banerjee (1993).

Entomologists are no strangers to the complexity that accompanies diversity, and are well positioned to make positive contributions to resolving some of the controversies that surround LB. In fact, entomologists have a vested interest in LB because many are potentially exposed to infection during fieldwork (Piacentino and Schwartz 2002; Vázquez *et al*. 2008) and yet are also aware of the potential for entomophobic hysteria. Furthermore, entomologists in Canada have an opportunity to make a fresh start, taking advantage of the currently low profile of the disease in Canada but building on the large volume of recent scientific studies from the USA and Europe. The purpose of this review is to explore the scientific, peer-reviewed research on LB that is relevant to Canada, with particular focus on biological and diagnostic factors that contribute to making this disease so challenging.

The natural history and ecology of LB

Diversity of *Borrelia* species and delimitation of *B. burgdorferi*

After investigating bacteria in ticks that were implicated in a cluster of juvenile arthritis cases from Old Lyme, Connecticut (41°19′N, 72°20′W), Burgdorfer *et al*. (1982) proposed that a spirochete was the etiologic agent of this illness. European researchers isolated a morphologically and immunologically similar spirochete from the skin and cerebrospinal fluid of a patient suffering from Bannwarth's syndrome (Pfister *et al*. 1984). The spirochete was formally named *B. burgdorferi* by Johnson *et al*. (1984) and later shown to include three distinct phyletic groups (Welsh *et al*. 1992). These three groups of strains, referred to as *B. burgdorferi sensu stricto* (s.s.), *B. garinii* Baranton *et al*., and *B. afzelii* Canica *et al*., were associated with differences in clinical symptoms within a broad definition of LB (Lebech *et al*. 1994). *Borrelia burgdorferi* tends to produce arthritic symptoms, *B. garinii* tends to infect neural tissue, and *B. afzelii* may persist in the skin (Terekhova *et al*. 2006; Tilly *et al*. 2008; Craig-Mylius *et al*. 2009; Hildenbrand *et al*. 2009; Kudryashev *et al*. 2009).

Rudenko *et al*. (2009) have now described *Borrelia carolinensis* from the southeastern USA as the 14th species of the *B. burgdorferi sensu lato* (s.l.) complex. The members of this species complex vary in host/vector

associations, pathogenicity, and distribution, and have now been recorded in many areas where they were not previously thought to occur (Rudenko *et al*. 2009). In North America, Lyme-like borreliosis may also be caused by *B. lonestari* Armstrong *et al*. (Stromdahl *et al*. 2003) and *B. bissettii* Postic *et al*. (Schneider *et al*. 2008), both of which occur widely on the continent. A Colorado isolate of *B. bissettii* has been shown to be pathogenic to mice, but an isolate from British Columbia was not (Schneider *et al*. 2008). Another species, *B. californiensis* Postic *et al*., is of unknown pathogenicity and has so far only been documented from California, where it is primarily associated with the California kangaroo rat, *Dipodomys californicus* Merriam (Postic *et al*. 2007).

Outside the *B. burgdorferi* s.l. complex, other *Borrelia* species can cause relapsing fever, with *B. hermsii* (Davis) vectored by argasid ticks (Ixodida: Argasidae) and occurring in British Columbia, Montana, and south through the Rocky Mountains (Schwan *et al*. 2007), and *B. recurrentis* (Lebert) vectored by the human body louse, *Pediculus humanus* L. (Anoplura: Pediculidae), in several widely separated Third World countries (Cutler *et al*. 1997). *Borrelia recurrentis* infection may occur either by crushing infected lice into broken skin or by contact with infected louse feces (Houhamdi and Raoult 2005). Difficulties in diagnosing *Borrelia* spp. have been reviewed by Exner (2004).

Unexpectedly large genetic diversity has also been found within *B. burgdorferi* s.s. For example, nine distinct clonal lineages were found at one field site in the northeastern USA (Bunikis *et al*. 2004). Differences in fitness between two of these genotypes, one isolated from blood and the other from skin, were studied in mice by Hanincová *et al*. (2008). The blood isolate retained its infectivity to xenodiagnostic ticks, whereas the skin isolate did not, suggesting partial diversification into more specialized subtypes in North America. Additional research in North America and Europe has shown further diversity in *B. burgdorferi* s.s.; at least 12 distinct sequence types defined by DNA sequence differences in outer-surface protein C (OspC) coexist in the northeastern USA and at least 17 OspC types are found across the USA and Europe (Qiu *et al*. 2008). *OspC-A* genotypes are associated with a highly virulent clonal group that has a wide distribution, suggesting recent migration of *OspC-A* genes into North America from Europe within the past few hundred years (Qiu *et al*. 2008). Interestingly, the *OspC-A* sequence at the *OspC* locus characterizes isolate B31, which was explicitly identified as the ''type strain'' in the original description of *B. burgdorferi* (Johnson *et al*. 1984). Most of the genome of B31 has subsequently been sequenced by Casjens *et al*. (2000).

Based on multilocus sequences for several slow-evolving housekeeping genes (MLSTs), Margos *et al*. (2008) agreed with Qiu *et al.* (2008) about the European origin of *B. burgdorferi*; however, they contended that *B. burgdorferi* has existed in North American refugia for much longer, in the order of millions of years. Although Margos *et al*. (2008) documented substantial discordance in topology as well as evolutionary processes between their concatenated MLST markers compared with the *OspC* locus, they did not consider whether recent introgression and hybridization of genes introduced from Europe may have contributed to the virulence of LB *via* hybridization. In other words, differences between MLSTs and OspC topology may reflect legitimately distinct biological processes in which housekeeping genes characterize a portion of the genome that has remained geographically stable, whereas *OspC* genes show signs of strong selection and recent trans-Atlantic gene flow. If so, this would mean that MLSTs may not unambiguously genotype *B. burgdorferi* samples or establish their evolutionary relationships and population structure at different levels, as was maintained by Margos *et al.* (2008).

Although intellectually fascinating, the complexity of *B. burgdorferi* s.l. has provoked spirited disagreement. Even the delimitation of LB is contentious. One school of thought is represented by the Infectious Diseases Society of America (Wormser *et al*. 2006, 2007) and relies on narrowly focused serology that is commonly applied in Canada using commercial

diagnostic kits such as MarDx (Canadian Public Health Laboratory Network (CPHLN) 2007), which were developed using the B31 strain of *B. burgdorferi* s.s. In contrast, the International Lyme and Associated Diseases Society defines LB more broadly as infection with *B. burgdorferi* s.l., which may be confounded with associated co-infections (Cameron *et al.* 2004). A small minority of practitioners even prefer to describe LB as a disease that is primarily a polymicrobial infection, emphasizing synergies of co-infections and host factors rather than the diversity of *Borrelia* spp. to explain the complex and notoriously variable clinical presentations of LB (Owen 2006). In common with other spirochetal diseases, LB has a wide spectrum of clinical symptoms that usually occur in successive stages (Miklossy 2008; Hildenbrand *et al.* 2009), and this diversity of symptoms, whether they are concurrent or successive, certainly contribute to the challenge of determining which of these symptoms are due to *B. burgdorferi*.

Vector incidence and distribution

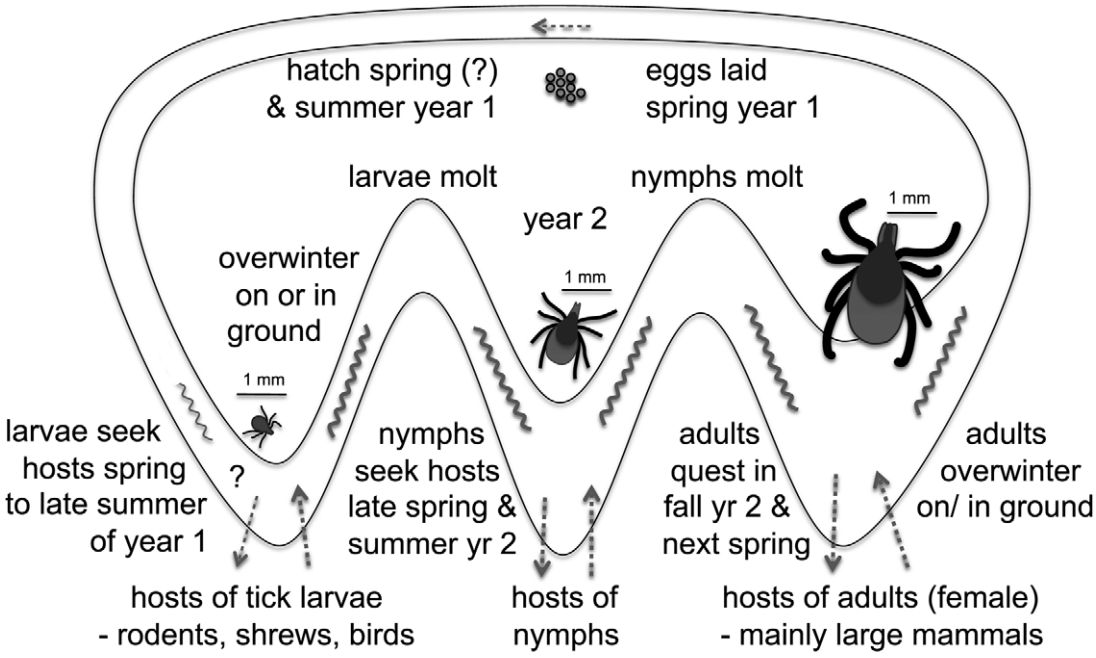
Borrelia burgdorferi is almost exclusively vectored by *Ixodes* ticks throughout its temperate Northern Hemisphere distribution (Eisen and Lane 2002). In British Columbia the primary vector is *I. pacificus* Cooley and Kohls, whereas across central and eastern Canada it is *I. scapularis*; together these two tick species are believed to be responsible for almost all human LB infections in Canada (Ogden *et al.* 2008*d*).

There are various methods of sampling ticks for study. Probably the most common is flagging (or dragging), which usually involves dragging a flannel sheet about 1 m^2 in area lightly against vegetation for a specific amount of time before inspecting it visually for adhering ticks (*e.g.*, Faulde and Robbins 2008; Ogden *et al.* 2008*d*; Scott *et al.* 2008). Alternatively, humans wearing protective clothing may be inspected for attached ticks (Lane *et al.* 2004; Faulde and Robbins 2008). These techniques suffer from the disadvantages that sampling takes place over a relatively short time period and is subject to weather conditions (Gray 1985).

Longer term sampling may be accomplished using caged sentinel animals (Burkot *et al.* 2001) or carbon dioxide traps (Gray 1985) but is problematic because *I. scapularis* has very limited horizontal movement (Falco and Fish 1991). Livetrapped animals can be inspected visually for ticks (Ogden *et al.*, 2008*c*; Salkeld *et al.* 2008) or their cages can be suspended over water into which the ticks fall (Ginsberg *et al.* 2005). Lane and Loye (1991) reported that visual inspection detected as few as 45% of the number of *I. pacificus* that were found using drop-off collection. Birds can be a source of ticks during banding (Scott *et al.* 2001; Morshed *et al.* 2005; Smith *et al.* 2006), as can deer killed by hunters (Lindsay *et al.* 1999*b*; Shariat *et al.* 2007). Passive surveillance may involve taking advantage of ticks removed in medical or veterinary clinics (Ogden *et al.* 2006, 2008*d*). A flaw of passive sampling is that the nymph is the tick life stage most likely to transmit disease to humans (Mather *et al.* 1996), but most ticks submitted as a result of passive surveillance are adult females, which are more easily detected (Morshed *et al.* 2006; Ogden *et al.* 2006).

The basic phenology of *Ixodes* ticks in Canada can be described relatively simply (Fig. 1) but there is substantial variation in the basic pattern within and between regions. An established population at Long Point in southern Ontario has received the best documentation to date (Lindsay *et al.* 1999*a*, 1999*b*). Larval activity in this area was lengthy, with weak peaks in mid to late June and mid-August (Lindsay *et al.* 1999*a*). On the other hand, nymphs were most abundant during a narrower time range, in June and July (Lindsay *et al.* 1999*a*), and adult activity was distinctly bimodal, with peaks in April and October (Lindsay *et al.* 1999*b*). The bimodal questing activity of adult *I. scapularis* in Ontario has been confirmed by Morshed *et al.* (2006), and in Manitoba, peak activity of adult *I. scapularis* occurs between September and November, with a second peak from April to June (Galloway 2002). Elsewhere in Canada the phenology of *Ixodes* may vary depending on local conditions; however, detailed studies are not generally available. In California, adult *I. pacificus* have a bimodal activity pattern, with peak

Fig. 1. Typical life cycle of the blacklegged tick, *Ixodes scapularis*, and associated infection by *Borrelia burgdorferi* in Canada, showing the succession of immunological and environmental challenges to spirochetes (broken lines and "wiggles") as they cope with tick searches for hosts, successive tick life stages, variation in blood-meal sources, and transmission to and from vertebrate hosts. Phenology is largely taken from Lindsay *et al.* (1999*a*, 1999*b*) and Galloway (2002), although the cycle may be extended for 1 or more years if ticks are unable to find food in any given year.



activity in both March and December and nymphs occurring between April and August (Clover and Lane 1995).

In agreement with Lindsay *et al.*'s (1999*b*) findings for southern Ontario, long duration and weak bimodality are also evident in the activity of larval *I. scapularis* from both the northeast and Upper Midwest of the USA (Gatewood *et al.* 2009), although the authors chose to fit a unimodal curve to their larval-occurrence data. In contrast, the seasonal activity of nymphs was explicitly recognized as bimodal, and the variation in size of the two nymphal-activity peaks was interpreted as the result of variation in the number of larvae that hatch in midsummer and then may or may not be able to feed before overwintering (Gatewood *et al.* 2009). However, it is plausible that adult phenology also contributes to the biomodality of both larval and nymphal activity. Extended larval activity may be due to the overlap of two cohorts: larvae hatching from eggs laid early in spring by females that had fed in the fall and overwintered, and those resulting from females that fed in spring and laid eggs somewhat later. As Killilea *et al.* (2008) have emphasized, there is a strong need for standardized sampling of all life stages of ticks across broad geographic regions.

Primary tick identification guides that are likely to be useful in Canada include Gregson (1956), Keirans and Clifford (1978), Furman and Loomis (1984), Durden and Keirans (1996), and Web sites such as *Ticks of Canada* (Canadian Lyme Disease Foundation 2009) and Anderson and Harrington (2009). Lindquist *et al.* (1999) produced couplets that augment the keys of Gregson (1956), Keirans and Clifford (1978), and Durden and Keirans (1996).

Established populations of *I. scapularis* have been well documented in localized areas of Nova Scotia, Ontario, and Manitoba

(Ogden *et al*. 2008*d*). Populations of *I. pacificus* have been established for at least a century in southern British Columbia (Gregson 1956). The dispersal and population structure of *I. pacificus* are still poorly understood, but work based on both allozymes and mtDNA has shown patterns of high local genetic diversity and locus-specific isolation or selection in localities ranging from West Vancouver, British Columbia, to southern California and Utah (Kain *et al.* 1997, 1999). *Ixodes* vectors can be spread by migratory birds across most of Canada (Scott *et al*. 2001; Morshed *et al*. 2005; Ogden *et al*. 2008*c*), which means that sporadic cases of LB can occur throughout Canada and transient populations of *Ixodes* ticks could establish in suitable localized habitats. Because *I. scapularis* and *I. pacificus* commonly feed on deer and other vertebrates that live in woodlands, increases in tick numbers can result from restoration of woodlands, especially in semi-urban areas, where hunting of deer is discouraged (Killilea *et al*. 2008). Changes in the distribution of ticks as a result of climate change are predicted to be disproportionately serious in Canada, with consequent increases in cases of LB (Ogden *et al*. 2006, 2008*a*, 2008*c*, 2008*d*).

Other confirmed vectors of *B. burgdorferi* that are known to range into Canada include *Ixodes angustus* Neumann, *I. dentatus* Marx, *I. jellisoni* Cooley and Kohls, *I. muris* Bishopp and Smith, and *I. spinipalpis* Hadwen and Nuttall (Gregson 1956; Eisen and Lane 2002). Older literature that lists *I. dammini* Spielman *et al*. as a vector of *B. burdorferi* (*e.g*., Lindsay *et al*. 1991) refers to *I. scapularis*; these two species are now considered conspecific (Wesson *et al*. 1993). *Ixodes cookei* Packard is not considered to be a competent vector of *B. burgdorferi* (Barker *et al*. 1993), although *B. burgdorferi* is documented from a specimen of *I. cookei* and its dog host in central Alberta (Fernando *et al.* 2008). *Borrelia garinii,* the most neurotropic of the European forms of LB, has been found in *I. uriae* White on seabirds in Newfoundland (Smith *et al*. 2006). In general, most *Ixodes* ticks in Canada appear to be competent, although not necessarily efficient, vectors of *B. burgdorferi* (Eisen and Lane 2002). *Ixodes gregsoni* has been newly described from mustelids in Canada Lindquist *et al*. (1999), but is unlikely to have much of an effect on transmission of *B. burgdorferi* because mustelids are poor reservoirs (LoGiudice *et al*. 2003). A number of ticks can acquire *B. burgdorferi* infections but are considered incompetent because they are unable to pass the infection on to the next host; these include *Amblyomma americanum* (L.), *Haemaphysalis leporispalustris* Packard, all species of *Dermacentor* C.L. Koch, and a few species of *Ixodes* (Eisen and Lane 2002). Vector competence is defined as the ability to (*i*) acquire spirochetes when feeding on an infected host, then (*ii*) pass them between tick life stages, and subsequently (*iii*) pass the infection to a susceptible host while feeding (Eisen and Lane 2002). Transmission of *Borrelia* from younger to older tick life stages is well established and such trans-stadial transmission is important because ticks generally feed on only one individual host per tick life stage (Oliver 1989). Less is known about the prevalence and importance of infection passed to larvae from adult females *via* the egg stage (Eisen and Lane 2002), although such transovarial infection may be important in Europe (Kurtenbach *et al*. 1995). For all forms of transmission, however, it is difficult to distinguish experimentally between complete incompetence and rare vectoring because of low tick infectivity (Kahl *et al*. 2002). The *B. burgdorferi*-vectoring competence of ticks is distinguished from mechanical transmission, where pathogens are not passed *via* the normal tick salivary secretions but are introduced past broken skin by a mechanism such as pathogens clinging to the mouthparts (an unlikely scenario considering the sensitivity of *Borrelia* to oxygen; Barbour and Hayes 1986) or, more plausibly, crushing a tick into a wound in the course of attempted removal. For a tick-borne proteobacterium, *Anaplasma* Theiler, in cattle, mechanical vectoring *via* the mouthparts of biting flies is approximately two orders of magnitude less efficient than biological vectoring (Scoles *et al*. 2005). However, the efficiency of mechanical vectoring of *Borrelia* and its role in human illness are not known. Until recently, autoinoculation by

crushing lice into wounds was thought to be the only way that *B. recurrentis* was transmitted (Houhamdi and Raoult 2005).

In addition to tick species, mosquitoes (Diptera: Culicidae), tabanid flies (Diptera: Tabanidae), and fleas (Siphonaptera) are known to harbor a competent vector *B. burgdorferi* but none has been shown to be competent as defined by Eisen and Lane (2002). In mosquitoes, for example, 7%–8% of adult female *Aedes* Meigen in Connecticut (Magnarelli *et al.* 1986) and 1.7% of *Culex pipiens* (L.) in Poland carried *B. burgdorferi* (Kosik-Bogacka *et al.* 2007). Furthermore, transmission between mosquito life stages is suggested by the occurrence of *Borrelia* in 1.6% of larval *Culex* L. in the Czech Republic (Zákovská *et al.* 2002) and 3.2% of larval and 1.6% of pupal *C. pipiens* in Poland (Kosik-Bogacka *et al.* 2007). Mites may also contain *B. burgdorferi* (Zakovska *et al.* 2008), but no surveyed spiders have been found to harbor *Borrelia* (Suffridge *et al.* 1999).

There are anecdotal reports of LB transmitted by vectors other than ticks in Canada (Doby *et al.* 1987) and the USA (*e.g.*, Luger 1990). These are unsupported by any of the evidence normally required to demonstrate competence or even mechanical vectoring. Nonetheless, although statements such as ''fleas, flies, and mosquitoes are not vectors for Lyme disease'' (Bratton *et al.* 2008) are common in the medical literature, it is conceivable that the detailed documentation of transmission by tick vectors has overshadowed occasional mechanical transmission by secondary vectors. Eisen and Lane (2002) state, ''Although insects may prove incompetent to serve as vectors for *B. burgdorferi s.l.*, the possibility of occasional mechanical transmission should not be discounted''. Further study is clearly needed.

Diversity and dynamics of reservoir hosts

The primary reservoir host of LB in North America has long been assumed to be white-footed mice (Levine *et al.* 1985), although recent research in the northeastern USA shows that two shrew species are each at least as likely as white-footed mice to be a potential source of infection for humans in some locations (Brisson *et al.* 2008). On the west coast of North America, infection rates demonstrate that squirrels are highly competent reservoirs and their infection rates correlate closely with rates of human infection (Salkeld *et al.* 2008). Major described hosts of *B. burgdorferi* that interact with humans include white-footed mice, chipmunks, squirrels, wood rats, and shrews (Killilea *et al.* 2008).

Some animals that act as common hosts for tick vectors, most notably deer in Europe and North America (Kurtenbach *et al.* 1998; Ullmann *et al.* 2003) and lizards in California (Lane 1990; Giery and Ostfeld 2007), are able to clear infection by *B. burgdorferi* and thereby may actively contribute to reducing rates of infection with *B. burgdorferi*. Thus, high deer densities could have both a positive and a negative influence on the zoonotic cycle of LB by allowing tick populations to be high while reducing rates of *Borrelia* infection in ticks if deer are the primary intermediate host between rodents, such as shrews, and humans. However, a recent report from Tennessee shows that 33% of winter ticks, *Dermacentor albipictus* (Packard), collected from white-tailed deer were positive for *B. burgdorferi* or *B. lonestari* (Shariat *et al.* 2007). The observation that *Borrelia* was not cleared from ticks feeding on these deer suggests that in some areas deer may still be significant reservoir hosts. The report by Shariat *et al.* (2007), however, is anomalous and it remains to be shown that *D. albipictus* could vector *Borrelia*. Lizards are also variable in their ability to clear *B. burgdorferi* infection (Clark *et al.* 2005) and are not universal in their incompetence as reservoir hosts (Giery and Ostfeld 2007).

Since birds, including migratory song birds that nest in urban habitats, are important for long-distance dispersal of ticks (Morshed *et al.* 2005; Ogden *et al.* 2008*c*; Scott *et al.* 2001) and some are important reservoirs for *B. burgdorferi* (Ginsberg *et al.* 2005), they can play an important role in peridomestic exposure to LB (Ginsberg *et al.* 2005).

In addition to variation in the strains of *Borrelia* that they tend to carry, reservoir animals exhibit differing symptoms of infection. For example, mice, commonly used as

models for human infection, do not develop the characteristic bull's-eye rash or exhibit clear neurological manifestations of LB, although they are excellent models for the arthritis form of LB (Yrjänäinen *et al*. 2007). This may explain the greater attention paid to the arthritis form of LB, for which an excellent animal model exists, than to the neurological form, where animal models are more problematic (Nardelli *et al*. 2008; Rupprecht *et al*. 2008).

Rates of infection of *I. pacificus* by *B. burgdorferi* are considered low in British Columbia (Ogden *et al*. 2008*d*) and reported as being less than 1% across the province (British Columbia Ministry of Health 2008). Charron and Sockett (2005) have speculated that this is due to the preference for lizard hosts shown by *I. pacificus* in the nymphal stage, presumably based on the borreliacidal properties of lizard blood demonstrated in California (Lane 1990; Slowik and Lane 2009). However the significance of such an interaction remains to be demonstrated in British Columbia, where lizards, being at the extreme northern edge of their ranges, are at much lower density. In British Columbia, the effect of climate change and the importance of patchiness in the distribution of *B. burgdorferi* and infected hosts are currently unpredictable (Ogden *et al*. 2008*d*).

Risk of human exposure to LB

The risk of human exposure to LB is related to numerous factors, such as the identity of ticks, the particular *Borrelia* strain, previous hosts, local ecological interactions, or human behavior (Horobik *et al*. 2006). Human behaviors that bring people into contact with ticks include sitting on logs, gathering wood, leaning against trees, walking in natural areas, and stirring or sitting on leaf litter (Lane *et al*. 2004). Even when considerable personal protection measures are taken by highly motivated entomologists, potentially infected ticks have been removed up to 2 days later, demonstrating that the risk of exposure to LB is still significant (Lane *et al*. 2004; Vázquez *et al*. 2008). Forest fragmentation may increase the risk of LB through loss of predators from smaller patches and a consequent increase in the abundance of small rodents, leading to higher densities of infected nymphal ticks (Allan *et al*. 2003). Also, highly diverse communities of vertebrates are less likely to bring infected nymphs into contact with humans, owing to "dilution effects" (LoGiudice *et al*. 2003). The dilution effect hypothesis states that when there are multiple vertebrate hosts for ticks, and these hosts differ in how well they clear infection but do not differ in resistance to ticks, then *B. burgdorferi* will no longer be transmitted as efficiently as when only the most susceptible hosts are infected. A number of other factors are also likely to influence the distribution and infection rates of ticks and there is a clear need for tick occurrence to be documented in a more standardized manner at multiple spatial and temporal scales (Killilea *et al*. 2008).

In the USA, the number of infected nymphs of *I. scapularis* and *I. pacificus* correlates with reported human disease more than does the incidence of adult ticks, and therefore nymphs are the life stage most commonly associated with disease transmission (Clover and Lane 1995; Mather *et al*. 1996; CDC 2007). The small size of *Ixodes* tick nymphs and their rapid feeding rate compared with that of adults are considered to be factors in increasing spirochete transmission to humans by nymphs (Clover and Lane 1995). Moreover, if nymphs are more likely than larvae to feed on hosts with borreliacidal blood, such as lizards or potentially even deer, this may also contribute to reducing rates of disease transmission by adult ticks relative to earlier life stages. Regardless of the underlying mechanism, it is important to survey nymphal ticks as well as adults to accurately assess disease incidence.

In regions of Canada where ticks are not endemic, however, the role of adults in vectoring should be clarified because, in such regions, a relatively high proportion of adults may have fed on migratory birds rather than on mammals when they were in the nymphal stage (Morshed *et al*. 2006). Birds from near Long Island, New York, have been demonstrated to be competent reservoirs for *Borrelia*, with wild-caught robins infecting 16% of larval ticks placed on them, whereas robins

that were previously infected by nymphal ticks in the laboratory passed that infection on to 82% of larvae that subsequently fed on them (Ginsberg *et al*. 2005). Although it is unclear whether similar infection rates apply to adult ticks that fed on birds as nymphs, it remains to be seen whether the adult stage of ticks may have greater importance in disease transmission in regions of Canada where *Ixodes* spp. are primarily derived from birds.

Physiological processes

Gene regulation and transmission dynamics

For *B. burgdorferi* to be transmitted from a cold-blooded tick vector to a warm-blooded host, it must regulate its gene expression in a complex and as yet incompletely understood manner. Models of gene regulation range from simple ones based solely on temperature and pH (Schwan *et al*. 1995) to a new paradigm suggested by Stevenson *et al*. (2006), who cautioned that the effects of temperature and pH may not predict the *in vivo* expression of genes. The synthesis of each of five different lipoproteins is controlled in a distinct manner, in some cases opposite to that predicted from temperature and pH effects *in vitro* (Stevenson *et al*. 2006).

Studies of the transmission dynamics of *B. burgdorferi* in *I. scapularis* indicate that the risk of transmission of strain B31 by a single bite from an infected tick is about 2% and that the risk increases with the length of time that the tick is attached (Hojgaard *et al*. 2008). When a tick first attaches, spirochetes are still found in the midgut and are producing outer-surface protein A (OspA), which helps spirochetes adhere to a midgut protein, TROPSA. When feeding begins, the spirochetes are exposed to warm mammalian blood and lowered pH, and OspA is downregulated while OspC is upregulated. Spirochetes then migrate from the midgut to the salivary gland and transmission to the vertebrate host can be achieved (*e.g*., Hojgaard *et al*. 2008). This delay in transmission explains why transmission is reduced when ticks are removed within 24 h of attachment (Hojgaard *et al*. 2008). In Europe, transmission of *B. burgdorferi* s.s. and *B. afzelii* by *I. ricinus* occurs in less than 24 h, but the risk of transmission still increases over time (Kahl *et al*. 1998; Crippa *et al*. 2002). In a further complication of the host–tick–pathogen interaction, *B. burgdorferi* s.l. is able to increase expression of an *Ixodes* salivary protein, Salp15, to protect against complement-mediated killing of *Borrelia* by the host's innate immune system (Ramamoorthi *et al*. 2005). This protective effect was greater when the vector was *I. ricinus* rather than *I. scapularis* (Schuijt *et al*. 2008). The early expression of *osp*C appears to be essential for *B. burgdorferi* to escape innate immunity and disseminate in the host (Gilbert *et al*. 2007), and yet persistent infection of the host is only possible when *osp*C is downregulated after infection because acquired antibodies to OspC allow the spirochetes to be cleared (Tilly *et al*. 2007). Current understanding of the interactions of tick saliva and *B. burgdorferi* is discussed in Anderson and Valenzuela (2007).

Even though vectoring by ticks is the most likely way to contract LB (and gene regulation of outer-surface proteins on *Borrelia* as well as increased expression of Salp15 in the tick vector will increase the probability of infection of the vertebrate host), there is nonetheless some evidence that contact transmission of *B. burgdorferi* can also occur without a vector. Burgess and Patrican (1987) have shown that deer mice can be infected orally by ingesting saline containing *B. burgdorferi* and that these mice can in turn infect ticks that feed on them. Despite an explicit search of the LB literature, it is not apparent that any studies have addressed whether *Borrelia* can be transmitted to vertebrate hosts that eat infected ticks or, for that matter, by contact between hosts *via* body fluids such as urine or sperm. However, Kurtenbach *et al*. (1994) have demonstrated differences in the antibody responses of various mouse species infected by needle inoculation *versus* tick bites. Tick-infected mice did not express antibodies to OspA or outer-surface protein B (OspB) early in the infection, whereas intradermally injected mice did (Kurtenbach *et al*. 1994). However, infection proceeded in both groups; in fact, intradermal inoculation is commonly used to ensure that a controlled quantity of pathogen is transferred in studies of LB

(*e.g.*, Hodzic *et al.* 2008). Recent research has shown that both OspA and OspB are involved in binding to the tick gut, so the results of Kurtenbach *et al.*'s (1994) study are counter to what would be expected. OspB-deficient *B. burgdorferi* is infectious and pathogenic in mice but is unable to survive in *Ixodes* (Neelakanta *et al.* 2007).

Careful and prompt removal of attached ticks is nonetheless important in reducing the probability of transmission of *B. burgdorferi* (Hojgaard *et al.* 2008). The preferred method is to grasp the mouthparts firmly with fine-pointed forceps and pull them straight out in a single action without twisting. This should reduce the risk of regurgitation of gut contents (Gammons and Salam 2002). An alternative method for removing attached ticks is the "straw and knot method" of Murakami (2009): a drinking straw is used to guide a thread to the base of the mouthparts, allowing the tick be pulled out in a single motion. Any remaining mouthparts should be excised and the area cleaned with antiseptic solution. Removal of the cement plug attaching the mouthparts to the wound may be important because *B. burgdorferi* can be present in it (Alekseev *et al.* 1995).

Persistent infection

Borrelia spp. are capable of persistent infection, and such persistence is the norm in mice, rats, hamsters, dogs, and monkeys (Barthold 2000; Straubinger 2000; Summers *et al.* 2005; Hodzic *et al.* 2008). Persistence in reservoir hosts can be interpreted as an evolutionarily shaped survival strategy linked to the asynchronous phenology of the tick vectors (Kurtenbach *et al.* 2006). Although natural infections are less controlled and defined, there is strong evidence that they persist in humans also (Oksi *et al.* 1999; Breier *et al.* 2001; Miklossy *et al.* 2004, 2008; Hunfeld *et al.* 2005). Immune evasion has been extensively documented in *Borrelia* (Liang *et al.* 2002; Bankhead and Chaconas 2007; Rupprecht *et al.* 2008; Xu *et al.* 2008*a*; Yang *et al.* 2009). Although *B. burgdorferi* is generally considered to be an extracellular pathogen, localization has been demonstrated within endothelial cells (Ma *et al.* 1991; Thomas *et al.* 1994), synovial cells (Girschick *et al.* 1996), and neuronal and glial cells (Livengood and Gilmore 2006; Miklossy *et al.* 2006). Infiltration of blood vessels, cardiac myocytes, and collagen tissues has also been demonstrated (Pachner *et al.* 1995), and adherence and escape of *Borrelia* from vasculature has recently been visualized directly by Moriarty *et al.* (2008). Sequestration and physical protection from the immune system in the extracellular matrix have been reviewed by Cabello *et al.* (2007) and Rupprecht *et al.* (2008).

When exposed to unfavorable conditions such as osmotic and heat shock, *B. burgdorferi* produces atypical forms (*e.g.*, Brorson and Brorson 1998, 2004; Singh and Girschick 2004; Miklossy *et al.* 2008; Margulis *et al.* 2009). The extracellular and intracellular presence of these atypical forms has been demonstrated in brain tissue of humans with confirmed neuroborreliosis, and atypical forms can exist in the absence of spirochetal forms (Miklossy *et al.* 2008). A thickened external membrane, observed by Miklossy *et al.* (2008) to surround cystic forms, was reactive to anti-OspA antibody and a full characterization of other immunogenic proteins was not attempted. Thus, a full understanding of the serology of LB must include the atypical forms as well as the typical spiral forms (Miklossy *et al.* 2008).

***Borrelia* has a unique, flexible genome**

Borrelia burgdorferi contains both circular and linear DNA molecules in roughly equal numbers (Rosa *et al.* 2005). Most replicons are linear molecules that are terminated by hairpin turns, which are unusual in bacteria and of much interest to molecular biologists (Chaconas 2005; Tourand *et al.* 2009). In addition to one large linear chromosome, *B. burgdorferi* has over 20 plasmids, the largest number so far described in any living organism (Chaconas 2005; Rosa *et al.* 2005) . Some of these plasmids carry crucial information for survival in tick vectors or mammalian hosts. The linear plasmid Ip28–1 is required for persistent infection with *B. burgdorferi* and the presence of the *vls* locus on this plasmid appears to be an absolute requirement for

persisting infection. Antigenic variation at *vlsE* by recombination of cassette fragments allows a large number of antigenically distinct variants to be produced, thus facilitating avoidance of the host's immune system (Bankhead and Chaconas 2007). The genetics of *B. burgdorferi* has been reviewed by Rosa *et al.* (2005) and Tilly *et al.* (2008). Furthermore, *Borrelia* spp. are unusual in having evaded the usual limiting factor of iron availability in the host cells by using manganese for electron transport (Posey and Gherardini 2000; Ouyang *et al.* 2009). Typically, at the onset of infection, humans secrete lactoferrin to limit available iron and inhibit bacterial growth (Arnold *et al.* 1982). *Borrelia burgdorferi* has eliminated most of the genes that encode proteins that require iron as a cofactor, and a manganese transporter is required for virulence in *B. burgdorferi* (Posey and Gherardini 2000; Ouyang *et al.* 2009).

Immune-system selection

Antigenic variation is a common mechanism whereby pathogenic bacteria can evade the immune system. *Borrelia* species are the subject of intense study that has revealed at least two different ways in which they achieve such antigenic variation. For *B. hermsii*, recombination of *vlp* and *vsp* sequences is responsible for antigenic variation, whereas in *B. burgdorferi*, recombination occurs at *vlsE* (Norris 2006). Furthermore, variation in VlsE of *B. burgdorferi* is not, by itself, sufficient to achieve resistance to reinfection, and is only part of a complex system that may confine the adaptive response of the host (Bankhead and Chaconas 2007).

Immune-system selection on *B. burgdorferi* has received extensive study and host specialization is thought to result from negative selection mediated by the complement system of the host's innate immune system (Schroeder *et al.* 2009). Spirochetes that are susceptible to destruction by the complement system of a given species are lysed early in the midgut of the tick (Kurtenbach *et al.* 2002*a*). This has led to the hypothesis that the host range of a spirochete strain is restricted by the genes that encode ligands that bind to complement inhibitors (Kurtenbach *et al.* 2002*b*).

The second main component of the mammalian immune system is the adaptive immune system, which introduces nonlinear frequency-dependent fitness, leading to fluctuations in the abundance of spirochete genotypes. Balancing selection by the adaptive immune system on the *ospC* gene maintains spirochete diversity within local tick populations (Schroeder *et al.* 2009), with persistent *B. burgdorferi* strains being favored over "boom and bust" ones (Kurtenbach *et al.* 2006). In a further refinement by Gatewood *et al.* (2009), persistent *B. burgdorferi* strains were interpreted as being favored when tick seasonality is asynchronous because of climatic conditions such as the relatively small difference between summer- and winter-temperature extremes in the northeastern USA. In the Upper Midwest of the USA, in contrast, less-persistent strains are favored because greater seasonal temperature disparities are associated with more overlap in the durations of larval and nymphal activity, allowing easier transmission of *B. burgdorferi* strains from infected nymphs to uninfected larvae (Gatewood *et al.* 2009). Modeling of tick phenology has been expanded to include synchronicity with reservoir hosts and photoperiod (Ogden *et al.* 2006, 2008*b*), and it is clear that further complexity remains to be modeled.

Diagnosis and serology of LB

Initial diagnostic steps

Although all evidence indicates that ticks are necessary for infection with *B. burgdorferi*, the ticks themselves are often not noticed. Steere (1989) reported that only 50%–70% of people report a tick bite before being diagnosed with LB. Tick saliva is well documented as containing an anaesthetic that reduces the probability of tick detection (Ribeiro and Francischetti 2003) and, considering their small size, immature ticks are likely to have an even lower rate of detection than adults.

On the other hand, knowledge of the presence of *Ixodes* ticks in an area can contribute to a diagnosis of LB. For example, an erythema migrans (EM) rash is considered sufficient by itself for a diagnosis of LB, but only in geographic areas where *I. scapularis* or

I. pacificus is established (CPS 2009). The presence of an EM rash, with its characteristic bull's-eye appearance, is not enough to justify treatment in areas of Canada where these *Ixodes* species are not believed to be endemic; in such cases confirmatory serology is advised before the diagnosis is given (CPS 2009). Variability in the presence and appearance of the EM rash has been extensively documented (Tibbles and Edlow 2007; Egberts *et al*. 2008). About 80% of confirmed LB patients are reported to show the rash (Aguero-Rosenfeld *et al*. 2005; Bratton *et al*. 2008), and American researchers often explicitly require the EM rash to be present for a patient to be considered for enrollment in a study (*e.g*., Wormser *et al*. 2008). Of 95 children presenting with facial palsy in an area of the USA where LB is endemic who later received serological confirmation of LB, only 35 reported the rash (Nigrovic *et al*. 2008). In Europe, only 18% of Swedish children with confirmed neuroborreliosis reported the rash (Skogman *et al*. 2008).

For a serological diagnosis of LB worldwide, either an enzyme-linked immunosorbent assay (ELISA) or an immunofluorescent assay (IFA) is expected to constitute the first step of a two-step diagnosis (Aguero-Rosenfeld *et al*. 2005). If the ELISA or IFA is positive, the second step is a Western blot immunoassay. Both the ELISA and the IFA are designed to detect serum antibodies produced by the host against proteins of *B. burgdorferi*. In Canada, only a positive or equivocal ELISA is currently accepted before a Western blot, which is more specific but more expensive, can be requested (CPHLN 2007). In the USA and Europe, either an ELISA or an IFA will fulfill the first step (CDC 1995; Brouqui *et al*. 2004). Like an ELISA, an IFA can use antibodies to whole-cell lysates or purified antigens such as flagellin, OspC, or P39. However, interpretation of an IFA requires well-trained personnel, whereas the more frequently used ELISA is more easily automated (Aguero-Rosenfeld *et al*. 2005). In Canada, the sensitivity of ELISA is considered to be close to 100% for tests performed 4 weeks post tick bite (Barker and Lindsay 2000; Forward 2005; Zaretsky 2006; CPHLN 2007; CPS 2009). This contrasts with Aguero-Rosenfeld *et al*. (2005), whose Table 4 reports that ELISA sensitivity is always less than 50% in the acute phase of an EM rash and only about 80% in the convalescent phase after antimicrobial treatment for an EM rash or a diagnosis of LB has been obtained because of neurological involvement. Only the arthritis form of LB was associated with higher ELISA sensitivity. There is considerable debate over the sensitivity of ELISA, especially in late stages of the disease (*e.g*., Donta 2007; Wilson 2007; Stricker and Johnson 2008).

In laboratory diagnoses, sensitivity is defined as the percentage of people truly suffering from a disease who are identified as positive by means of an assay (Saah and Hoover 1997). This is not the same as the more commonly understood use of the term sensitivity for analyses, which refers to an assay's ability to detect a low concentration or signal in a sample (*e.g*., the ability of an ELISA to detect low quantities of antibodies to *B. burgdorferi*). Diagnostic specificity, on the other hand, is defined as the percentage of people who do not have a disease and who are identified as negative by means of an assay (Saah and Hoover 1997). High diagnostic sensitivity means that an assay produces a high rate of true positives and a low rate of false negatives. Furthermore, high diagnostic specificity means a high rate of true negatives and a low rate of false positives. Laboratory diagnosis of LB has tended to emphasize diagnostic specificity and the need to eliminate false positives, rather than high sensitivity and the elimination of false negatives (CPHLN 2007; CPS 2009). Thus, a patient may eventually be diagnosed as having LB on the basis of PCR tests, observations of spirochetes in tissue, or clinical symptoms, in spite of an earlier ELISA test that returned a negative result. Clinicians, however, are often not aware of the potential for such false negatives, especially in late disease (Donta 2002, 2003, 2007; Wilson 2007).

Because ELISA is used as a screening method in Canada (CPHLN 2007), a negative ELISA may be taken as evidence that there is no infection with *Borrelia*. This interpretation of ELISA has resulted in the claim that there

is no valid support for the idea that LB is underdiagnosed in Canada (CPS 2009). This viewpoint contrasts with Ogden *et al*. (2008*d*), who stated that underreporting is more biologically plausible. The documented genetic diversity and biological complexity of *B. burgdorferi* s.l. cannot help but contribute to difficulties in detecting *Borrelia* with both high sensitivity and high specificity, especially when the spirochetes enter tissues, such as collagen, to which the host's immune system has limited access (Cabello *et al*. 2007; Miklossy *et al*. 2008).

Effectiveness of specific ELISA tests

Commercial ELISA kits can be based on whole-cell sonicates or more specific antigens like flagellin and C6 peptide. Currently, whole-cell sonicates of strain B31 are used in Canada for humans (CPHLN 2007), whereas C6 peptide kits (IDEXX Laboratories, Inc. 2008) are favored for dogs. The C6 peptide ELISA relies on a single peptide from the VlsE protein, a lipoprotein with an invariable region, C6, which is highly antigenic. Because the assay based on C6 peptide is different from the standard ELISA used for the same organisms, dogs may serve as sentinels for some *B. burgdorferi* s.l. strains that are not detected using the whole cell sonicate ELISA kits used for humans.

The use of dogs as sentinels for LB is controversial and has recently been reviewed by Fritz (2009). The C6 peptide assay is considered to allow greater specificity than earlier assays, since it is only expressed in actively infected animals (Liang *et al*. 1999, 2002). Although treatment of seropositive dogs is discouraged unless clinical signs are present, the use of domestic dogs to demonstrate the presence of *B. burgdorferi* in areas where ticks are localized may lead to an underestimate rather than an overestimate of the presence of *B. burgdorferi* (Fritz 2009).

Some researchers use an in-house sonicate derived from strain B31 (*e.g*., Steere *et al*. 2008), whereas others use whole-cell sonicates of N40 (*e.g.*, Pachner *et al*. 2002). Wormser *et al*. (2008) maintained that the diversity of LB strains is unlikely to be relevant to the diagnosis of human disease provided a whole-cell sonicate is used in the ELISA. However, use of whole-cell sonicates produces a high level of background absorbance, necessitating correction for false positives and potentially reducing the detection of true positives (Lawrenz *et al*. 1999; Donta 2002). It has been suggested that this effect explains the low sensitivity in a serological survey of deer in Ontario (Gallivan *et al*. 1998). Wilske *et al*. (2007) recommend that because of the heterogeneity of causative agents of LB in Europe, ELISAs should be based on OspC as well as DbpA, VlsE, or C6 peptide to improve sensitivity.

Commercial ELISA kits licensed for use in the USA vary substantially in sensitivity. Aguero-Rosenfeld *et al*. (2005) reported ranges from 33% (EM rash) to 79% (neuroborreliosis) and 100% (Lyme arthritis). Such an ascertainment bias in favor of the arthritis form of LB may account for statements that Lyme arthritis, the original name of the disease, is the most common form of late LB in North America (Wormser *et al*. 2006, 2007). Furthermore, the B31 strain has been shown to cause arthritic symptoms disproportionately relative to other members of *B. burgdorferi* s.l. (Terekhova *et al*. 2006; Tilly *et al*. 2008; Craig-Mylius *et al*. 2009; Hildenbrand *et al*. 2009; Kudryashev *et al*. 2009), so it is not unexpected that serological testing based on B31 is particularly effective in detecting the arthritis form of LB.

Western blots

Western blots, also called immunoblots, use gel electrophoresis to separate proteins by size or shape, after which they are visualized by staining with antibodies specific to the target proteins and scored for presence and intensity of the banding pattern. They are generally more expensive to run than an ELISA because they are more labor-intensive and require highly trained personnel to interpret the banding patterns (Bjerrum and Heegaard 2001).

A Western blot of patient serum antibodies to LB antigens is required as the second step in a serological diagnosis of LB. In Canada, the Western blot must have at least 2 of 3 bands for immunoglobulin M (IgM) as well as at least 5 of 10 bands for immunoglobulin G

(IgG) (CPHLN 2007). These criteria were established for active or previous infection and were designed to identify a banding pattern that gave high statistical specificity. Bands for IgM antibodies to both OspA and OspB and for IgG antibodies to OspB were explicitly excluded (Dressler *et al*. 1993), even though these bands are highly diagnostic. Because these proteins were intended as candidates for vaccine development (Fikrig *et al*. 1992), assays based on these bands would have been unable to distinguish between active LB and vaccination. An OspA vaccine was eventually released but withdrawn in 2002, ostensibly for economic reasons but there were suggestions that it had autoimmune effects (Rosé *et al*. 2001; Nigrovic and Thompson 2007; Nardelli *et al*. 2008).

It is generally accepted that OspC is the immunodominant protein involved in the IgM response, while decorin-binding protein A (DbpA) is the immunodominant protein in the IgG response (Jovicić *et al*. 2003; Wilske *et al*. 2007). However, Fingerle *et al*. (1998) demonstrated that 31% of *B. burgdorferi* from ticks that had been feeding on humans in southern Germany expressed OspA rather than OspC and that these *B. burgdorferi* were capable of causing disease in humans. Some current Western blot kits used in Canada, such as MarDx (CPHLN 2007), are based on a sonicate of strain B31. False-negative serology is considered a significant risk in neuroborreliosis unless multiple *Borrelia* strains are tested for (Kaiser 2000; Jovicić *et al*. 2003). In Europe, multiple *Borrelia* species are acknowledged and Western blots are interpreted differently than in North America, where *B. burgdorferi* s.s. is often assumed to be responsible for all human LB (CDC 1995; Robertson *et al*. 2000; Wormser *et al*. 2006, 2007; Vanousová and Hercogová 2008).

To confirm a LB diagnosis, it may be considered necessary to document conversion from IgM to IgG about 4 weeks or more after infection (Aguero-Rosenfeld *et al*. 2005; Wormser *et al*. 2006, 2007). In some people, however, seronegativity with only a cell-mediated immune response is possible (Singh and Girschick 2004). In addition, detection of antibodies that are tied up in complexes would be missed by standard tests that rely on free antibodies (Singh and Girschick 2004; Holl-Wieden *et al*. 2007).

Antigenic variation

Preferential expression of proteins by *B. burgdorferi* in both the tick vector and the mammalian host results in differential expression of antibodies that can be detected by immunoblots. For example, OspA/B is expressed in ticks (Neelakanta *et al*. 2007), whereas dmpA/B and bmpA/B are expressed in the mammalian host (Pal *et al*. 2008; Shi *et al*. 2008). The serological response in early LB would be expected to include proteins that may be downgraded in later disease while the antibody response to later disease would be expected to show different proteins. As with vlsE, antigenic variation within a single immunogenic protein allows *B. burgdorferi* to evade the host response and so immunblots would be expected to allow only variable detection of some proteins. Fortunately, multiple immunogenic proteins have been described and in recent work a genome-wide proteome array and protein microassay have been used to expand the number of diagnostic antigenic proteins that are useful in serological diagnosis (Barbour *et al*. 2008; Xu *et al*. 2008*b*).

PCR assays

North American practitioners have been reluctant to accept PCR tests as the primary basis for a diagnosis of LB (*e.g*., Halperin and Wormser 2001). Such tests can show a large variation in specificity among studies, tissue categories, and geographic regions. Meta-analysis of PCR assays carried out in North America and Europe generally show moderate median sensitivity for skin biopsy (69%) and synovial fluid (78%) but low median sensitivity for cerebrospinal fluid (38%) and blood/plasma/serum (14%) (Aguero-Rosenfeld *et al*. 2005). The ranges of these values were at least 50% among individual studies, and when the studies were grouped by continent the difference in median sensitivity between North

America and Europe ranged from 8% (blood/plasma/serum) to 50% (cerebrospinal fluid). However, specificity rates for these categories are much higher, usually 100%, with the lowest being 93% (Aguero-Rosenfeld *et al.* 2005), which means that there are very few false positives. Thus, in spite of extensive documented variation in specificity and high rates of false negatives, PCR tests can be useful for confirming clinical diagnoses in individual cases. For example, a case of seronegative Lyme arthritis has been described where PCR-positive synovial fluid allowed a diagnosis to be made in the absence of a serological response (Holl-Wieden *et al.* 2007).

Although PCR-based diagnosis of LB in humans is problematic, presumably because spirochetes can be sequestered in tissues that were not tested, this is not such an impediment to the detection of *Borrelia* in ticks. Numerous studies have consistently demonstrated that whole or partial ticks can be homogenized to give sufficient DNA for identifying a substantial variety of bacterial symbionts (*e.g.*, van Overbeek *et al.* 2008). Furthermore, DNA-based identification of the ticks themselves is feasible *via* DNA barcoding or other DNA-based techniques (Sperling and Roe 2009), which should be especially useful for identifying immature stages, where diagnostic characters visible in their morphology are limited.

Future directions

Human cases of LB in Canada can be devastating to affected individuals, not only because of increasingly debilitating health effects over time, but because of the psychologically draining process of obtaining a formal diagnosis that allows treatment *via* the Canadian health system. It is easy to understand why a diagnosis of LB can be difficult to obtain in the face of its ecological complexity, genetic diversity, and immunological heterogeneity. With so much specialized information to assimilate and comprehend, it can be equally difficult to know where efforts to improve the situation can make a significant difference. Fortunately, it is likely that entomologists can contribute in a number of ways to a positive resolution of the challenges presented by LB.

First, there is a strong need for better means of identifying tick species and for detailed documentation of their distributions across Canada. Morphology-based keys that require only the use of a good microscope have been available for many decades, but relatively little new work on publicly accessible identification guides for ticks in Canada has been done since Gregson (1956). However, a handbook to the ticks of Canada is nearing completion (T. Galloway, University of Manitoba, Winnipeg, personal communication) and should fill an urgent need. Furthermore, there is good potential for DNA-based identification and delimitation of tick species (Sperling and Roe 2009), and internet-accessible guides can supplement these efforts. Such efforts should facilitate localized surveys of tick distributions, which are essential to more effective modeling and prediction of changes in tick and LB distributions in the context of climate change (Ogden *et al.* 2008*a*, 2008*c*, 2008*d*). The patchy distributions of *B. burgdorferi*-infected hosts and ticks means that using average numbers of infected ticks across a large area may underestimate the prevalence of LB. Efforts to identify patches should focus on areas where exposure of humans or their companion animals has been reported, because large-scale inventories of ticks are unlikely to pick up infrequent patches. The dispersal and population structure of ticks can be studied using molecular markers such as microsatellites, and the field is ripe for further work of this kind (*e.g.*, Kain *et al.* 1999).

The second way in which entomologists can contribute, in conjunction with microbiologists, is through more effective identification of *Borrelia* species and other symbionts in ticks. This will allow more effective study of the factors that regulate the behavior and interaction of ticks with their vertebrate hosts and bacterial symbionts. PCR-based diagnostics of *Borrelia* species and surveys of the diversity of bacterial strains in ticks are now commonplace. These should enable more comprehensive documentation of the distribution and evolution of these symbionts in

spite of the challenges of growing them in culture (Aguero-Rosenfeld *et al*. 2005). Furthermore, new techniques for *in vivo* visualization of *Borrelia* are providing insights into the behavior of spirochetes (Moriarty *et al*. 2008). Round bodies of *B. burgdorferi* have also been visualized post mortem in confirmed cases of LB (Miklossy *et al*. 2008); this method may therefore assist in confirming previously inconclusive cases.

Consistent identification of *Borrelia* cannot help but increase our understanding of the transmission dynamics of these bacteria in specific ecological or behavioral circumstances. For example, Faulde and Robbins (2008) recently demonstrated that fewer infected ticks were picked up by dragging for the main European vector of LB, *I. ricinus*, than by collecting from exposed human volunteers, which suggests that *Borrelia burgdorferi* s.l. may increase the host-finding efficacy of female *I. ricinus* under natural conditions. Such an effect may explain why some of the low numbers of ticks collected in Canada by dragging or flagging test positive. It also supports the use of passive surveillance, because a higher percentage of positive ticks would be expected from ticks collected on hosts. Furthermore, *Borrelia* incidence and vector competence should be addressed more comprehensively in tick species other than those of *Ixodes*. An example is the competence of deer and other ungulates as reservoirs for *B. burgdorferi*. Even for *Ixodes* ticks, little is published that gives deer infection rates in Canada beyond the studies done in Ontario by Gallivan *et al*. (1998). Although the finding obviously needs to be confirmed, the Tennessee study by Shariat *et al*. (2007) opens up the possibility that deer are effective reservoir species in some areas, and *Dermacentor* ticks may even be involved as vectors.

A third major research gap in Canada is the synergistic effect of co-infections in vectors or alternative reservoirs, which opens up opportunities for entomological collaboration with public health researchers as well as with microbiologists. The diversity of species and strains of *Borrelia* is still incompletely understood and the most important combinations involved in these co-infections are largely unknown. Simultaneous infection by *B. burgdorferi* and *Babesia microti* Franca increases the severity of arthritis in mice (Moro *et al*. 2002). Human infection with both *B. burgdorferi* and *Babesia microti* was described in Ontario in 1997, with the recognition that more serious disease symptoms are associated with co-infection (dosSantos and Kain 1998). More severe symptoms continue to be reported for LB cases with co-infections (Rawling *et al*. 2009). For vectors, Zhong *et al*. (2007) have demonstrated that in *Amblyomma* C.L. Koch ticks treated with antibiotics, reproductive fitness is reduced. In their study system, *Coxiella* sp. Philip was the identified endosymbiont. Because *I. scapularis* also has an endosymbiont, a *Rickettsia*-like bacterium (Noda *et al*. 1997), this information may have relevance to the LB system. Not only would treating the ticks with antibiotics potentially reduce the incidence of *B. burgdorferi*, it might also reduce the population of the tick vector. The effectiveness of antibiotic-baited traps that target mice (Dolan *et al*. 2008) may have further advantages for integrated pest management strategies for controlling LB by lowering populations of *Ixodes* in subsequent years.

Practitioners of integrated pest management and other aspects of economic entomology will recognize a fourth research need: a long-term and broad-scale analysis of the costs and challenges of LB to humans, which is essential to a more effective deployment of resources to combat this disease. Recognizing that human cases are possible across the geographic expanse of Canada is vital to reducing the burden on the public health care system. Furthermore, recognizing and monitoring the changing effects of LB over time will pay dividends on a human scale. In Scotland the cost of treating a case of early LB is estimated to be one-third that of treating late LB (Joss *et al*. 2003), and early and effective treatment is viewed as a cost-saving measure. In Scotland, regular audits of the interpretation of Western blots that are tailored to local areas has been emphasized. This has resulted in improved management of patients, and it is now recognized that the 58-kilodalton protein is a highly specific indicator of infection by *B. burgdorferi*

(Evans *et al.* 2005). A revised antigen for IgG Western blotting has been produced and has increased sensitivity for Scottish patients (Mavin *et al.* 2009). Canadians might do well to follow this example and conduct an audit of the proteins used to establish a positive Western blot. The various ELISA tests are also in need of renewed scrutiny.

Variability and recurrence of symptoms are hallmarks of LB (Cameron *et al.* 2004; Holl-Wieden *et al.* 2007; Nardelli *et al.* 2008). Establishment of the initial infection, its dissemination, and the development of pathology are all stages in this complex disease. Debate is ongoing concerning the severity of symptoms and the role of persistent infection and immunological dysfunction in continued symptoms. The ecology of LB is challenging: at every spatial scale studied so far, the risk is unevenly distributed (Killilea *et al.* 2008). A coordinated, multidisciplinary, and flexible approach to understanding LB is needed to reduce the burden of this disease in Canada and abroad. Entomologists are in an ideal position to achieve rapprochement between the highly divergent groups of people concerned with LB and to contribute to a better understanding of its practical and intellectual challenges.

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